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The applicant has filed a statement in accordance with Rule 28 (4) EPC (issue of a sample only to an expert). Accession number(s) of the deposit(s): DSM 3819, DSM 4109, DSM 4110, DSM 4111.

(54) **Enzymatic detergent additive.**

(57) Lipase is derived from Humicola sp. (incl. Thermomyces sp.), preferably H. lanuginosa. This lipase is found to have high activity at alkaline pH and to be compatible with anionic surfactants, and it is more effective as a detergent additive than prevously described detergent lipases.

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Description

ENZYMATIC DETERGENT ADDITIVE

TECHNICAL FIELD

This invention is related to an enzymatic detergent additive the active component of which is a microbially produced lipase, to a detergent comprising such additive and to a washing process using such detergent.

BACKGROUND OF THE INVENTION

The field comprising enzymatic additives in detergents has been rapidly growing during the last decades. Reference is made to e.g. the article "How Enzymes Got into Detergents", vol. 12, Developments in Industrial Microbiology, a publication of the Society for Industrial Microbiology, American Institute of Biological Sciences, Washington, D.C. 1971, by Claus Dambmann, Poul Holm, Villy Jensen and Mogens Hilmer Nielsen, and to P. N. Christensen, K. Thomsen and S. Branner: "Development of Detergent Enzymes", paper presented on 9 October 1986 at the 2nd World Conference on Detergents held in Montreux, Switzerland.

Proteolytic detergent additive is widely used in Europe, USA and Japan. In several countries, the majority of detergents, both powder and liquid, contain protease.

The use of lipase as a detergent additive is known. For a comprehensive review we refer to H. Andree et al.: "Lipases as Detergent Components", Journal of Applied Biochemistry, 2, 218-229 (1980). Further examples may be found in US 4,011,169 (column 4, line 65 to column 5, line 68), in GB 1,293,613 (page 2, lines 6-29) and in the paper by T. Fujii entitled "Washing of Oil Stains with Lipase" (in Japanese) given at the 16. Symposium on Washing, held in Tokyo on September 17-18, 1984.

Among the known lipases used as detergent additives, to the best of our knowledge the Fusarium oxysporum lipase has the best lipolytic characteristics, looked upon from a detergent application point of view, vide our European patent application with publication No. 0 130 064, especially the comparative table on page 27.

If the washing process is conducted at high temperature and high alkalinity, the majority of the fat containing dirt will be removed anyway. However, low or medium temperature washing processes (around 60°C and below) are now generally used, and at these low temperatures the known lipases are able to dissolve only a small part of the fat containing dirt.

Hitherto the efficiency of lipolytic detergent additives usually has been measured by washing of EMPA (Eidgenössische Materialprüfungs- und Versuchsanstalt, St. Gallen, Switzerland) swatches Nos. 101 (olive oil/cotton) and 102 (olive oil/wool) by adaptation of the procedure described in British patent No. 1,361,386 (especially pages 4 and 7) and US patent No. 3,723,250 (especially col. 15 - 19). In this way lipolytic cleaning efficiency can be expressed as the differential reflectance value Δ R. However, two more direct measures of the lipolytic action were used here. First, the weight of oil remaining on the textile was determined; this shows the combined effect of detergent and lipase. Second, the remaining oil was analyzed for oil (triglyceride) and decomposition products (mono- and diglyceride, and fatty acid), and the number of unhydrolyzed glyceride bonds in the oil was calculated; this shows more directly the effect of lipase. By use of these latter determinations it has been found that even the best known detergent lipase exhibits a lipolytic detergency effect which is open to improvement.

Furthermore, it is common knowledge that lipases, being proteins, are liable to attack by proteases, and as mentioned above, proteases are today contained in many detergents. There is no publication of a detergent lipase having satisfactory stability in the presence of protease. In fact, we have found that some known detergent lipases have poor stability in detergent solutions in the presence of commonly used detergent proteases.

Thus, a need exists for a lipolytic detergent additive which exhibits a considerably better lipolytic detergent efficiency at economically reasonable lipase activities in the washing solution and which is stable in detergent solutions containing detergent protease.

STATEMENT OF THE INVENTION

The first aspect of the invention provides a lipolytic detergent additive characterized by the fact that the lipase is producible by means of a strain of the genus Humicola, incl. the genus Thermomyces.

In another aspect, the invention provides a lipolytic detergent additive characterized in that the lipase cross-reacts immunologically with the lipase from Humicola lanuginosa strain DSM 3819.

Further aspects of the invention provide a detergent comprising the above-mentioned lipolytic detergent additive and a washing method using this detergent at pH 7-12.

The detergent lipases of the invention shows a superior detergency compared to previously known detergent lipases. Further, the lipases used in the invention are stable in detergent solution in the presence of commonly used detergent proteases, in contrast to known detergent lipases.

It is described in Japanese unexamined patent publication No. 48-62990 that Humicola lanuginosa is a lipase producer. However, this Japanese patent publication fails to suggest that the H. lanuginosa lipase is suited as an active component in an enzymatic detergent additive. On the contrary, it appears from fig. 1 in the Japanese patent publication that the pH-optimum of H. lanuginosa lipase is around 8, and that the activity declines

sharply when the pH value increases above 8. Thus, it would be expected that this lipase is unsuited as a detergent additive, as the pH in washing solution is usually far above 8. Surprisingly, however, we have found that the H. lanuginosa lipase has a pH optimum far above 8, vide Example 1 later in this specification.

Also, it is described in Current Science, August 5, 1981, Vol. 50, No. 15, page 680 that H. lanuginosa lipase can be used in dry cleaning. As the pH optimum, which exclusively relates to aqueous media, is of no significance whatsoever in relation to a dry cleaning lipase, this statement is not relevant to a possible suitability of the H. lanuginosa lipase as a lipolytic detergent additive.

Furthermore, it appears from Agr.Biol.Chem. 37 (11), p. 2488 (1973) that H. lanuginosa lipase is strongly inhibited by addition of certain anionic surfactants. However, we have found that surprisingly H. lanuginosa lipase is excellently compatible with LAS, a commonly used anionic surfactant.

DETAILED DESCRIPTION OF THE INVENTION

Lipase-producing microorganisms

Lipases of the invention are preferably obtainable from strains of thermophilic Humicola sp., including thermophilic Thermomyces sp., such as H. lanuginosa (Griffon and Maublanc) Bunce, H. stellata Bunce, H. grisea var. thermoidea, Cooney & Emerson, H. insolens, Cooney & Emerson, Thermomyces ibadanensis, Apinis & Eggins, H. hyalothermophila Moubasher, Mazen and Abdel-Hafez, H. grisea var. indica Subrahmanyam, H. brevis var. thermoidea Subrahmanyam and Thirumalachar and H. brevispora Subrahmanyam and Thirumalachar.

In a specially preferred embodiment of the enzymatic detergent additive according to the invention the lipase is producible from H. lanuginosa (Griffon and Maublanc) Bunce, H. brevispora Subrahmanyam and Thirumalachar, H. brevis var. thermoidea Subrahmanyam and Thirumalachar or H. insolens Cooney & Emerson.

H. lanuginosa has also been described under the synonyms Thermomyces lanuginosus Tsiklinsky, Sepedonium lanuginosum Griffon and Maublanc, Sepedonium thermaphilum cyclosporum and S. thermaphilum ovosporum Velich, Acremoniella sp. Rege, Acremoniella thermophila Curzi and Monotospora lanuginosa (Griffon and Maublanc) Mason.

Moreover, the species Scytalidium thermophilum (Cooney & Emerson) Austwick was by Hedger (1975, The ecology of thermophilic fungi in Indonesia. In biodegradation et Humification. Rapport due ler Colloque International - Nancy 1974 (ed. G. Kilbertius, O. Reisinger, A. Mourey & J. A. Cancela Da Fonseca), Sarreguemines: Pierron Editeur - 57206) considered as belonging to Humicola insolens.

In a particularly preferred embodiment the lipase is producible from one of the following strains:

taxonomic designation	internal No.	deposit No.	deposit date
H. lanuginosa	A 1231	DSM 3819	13 Aug 1986
H. lanuginosa	H 126	DSM 4109	4 May 1987
H. brevispora	A 2121	DSM 4110	4 May 1987
H. brevis var. thermoidea	A 2106	DSM 4111	4 May 1987
H. insolens	C 579	DSM 1800	1 Oct 1981

DSM indicates Deutsche Sammlung von Mikroorganismen. The strains have been deposited under the terms of the Budapest Treaty.

Lipase for use in the invention may be produced by aerobic cultivation of one of the above strains according to principles known in the art, e.g. as the examples given later.

Immunochemical characterization of lipases

It is to be understood that lipases produced by genetic engineering on the basis of Humicola sp. and with active centers identical to the active centers of the lipases producible from Humicola sp. are also within the scope of this invention. The preferred lipases of the invention cross-react immunologically with (are antigenically identical or partially antigenically identical to) a lipase from Humicola sp., more particularly with the lipase from one of the above-mentioned species, particularly H. lanuginosa and especially from one of the above-mentioned strains, notably DSM 3819 and DSM 4109.

The identity (cross-reaction) tests can be performed by the well-known Ouchterlony double immunodiffusion procedure or by tandem crossed immunoelectrophoresis according to N.H. Axelsen: Handbook of Immunoprecipitation-in-Gel Techniques (Blackwell Scientific Publication, 1983), Chapters 5 and 14. The terms "antigenic identity" and "partial antigenic identity" are described in the same book, Chapters 5, 19 and 20.

Using monospecific rabbit antiserum raised against purified lipase from DSM 4109, we found that the lipases

from strains DSM 3819, DSM 4109, DSM 4110 and DSM 4111 are all antigenically identical by both of the above-mentioned methods. Production of antiserum is described in N.H. Axelsen's book, Chapter 41. Purification of lipase is described in W-H Liu, Agr. Biol. Chem., 37(1), 157-163 (1973); however, we found that the column chromatography may be more conveniently performed by use of: DEAE-sepharose (anion exchange chromatography), phenyl sepharose (hydrophobic interaction chromatography), followed by gel filtration on TSK G3000SW.

Enzymechemical characterization of lipases

The pH dependence of the activity was determined by a traditional method, using tributyrine as substrate at 30°C in a pH stat and with gum arabic as emulsifier. The activity at various pH was found from alkali consumption versus time.

pH dependence was also checked with a more realistic substrate, viz. olive oil adsorbed on PVC (according to US patent 4,284,719).

pH-activity curves for lipase from H. lanuginosa DSM 3819 are shown in fig. 1 (tributyrine) and fig. 2 (olive oil/PVC). The curves for DSM 4109, DSM 4110 and DSM 4111 were very similar, showing optimum at pH 10.0 - 10.5 by both methods. pH-activity curves for lipase from H. insolens DSM 1800 are shown in fig. 3 (tributyrine) and fig. 4 (olive oil/PVC).

Isoelectric focusing was performed on the five lipases, followed by a tributyrine overlayer to detect lipase activity. It was found that DSM 3819, DSM 4109, DSM 4110 and DSM 4111 all have lipase activities with pI around 4.5, while DSM 1800 has the main part of its lipase activity with pI around 9.0 - 9.5 and only a minute amount of the lipase activity with pI around 4.5.

Detergent additive

In a preferred embodiment, the enzymatic detergent additive according to the invention is provided as a non-dusting granulate or as a liquid. These are suitable for use in powder detergents and liquid detergents, respectively. Granulates can be produced in several different ways. Reference can be made to GB patent No. 1,362,365 which describes the production of enzyme containing granulates used as detergent additives by means of an apparatus comprising an extruder and a spheronizer (sold as MARUMERIZER®), and to US patent No. 4,106,991 which describes the production of enzyme containing granulates used as detergent additives by means of a drum granulator.

In the case of a liquid formulation, storage stability tends to be unsatisfactory, and a liquid with an enzyme stabilizer is therefore preferred. The stabilizer can be propylene glycol or other agents known as stabilizers for enzyme solutions. As will be shown later in this specification, a straight aqueous solution of the lipase of the invention has poor storage stabilty, but this can be remarkably improved by the inclusion of stabilizers, e.g. propylene glycol.

In a specially preferred embodiment of the enzymatic detergent additive according to the invention, the lipase activity is above about 10,000 LU/g of additive. Lipase Unit (LU) will be defined later in this specification. In this manner, a convenient lipase activity is generated in the washing solution when the detergent additive is added to the detergent in an amount of 0.1 to 5.0 g/100 g of detergent, and when the detergent is added to the washing solution in an amount of 0.5 -20 g of detergent/l of washing solution.

In a specially preferred embodiment, the enzymatic detergent additive according to the invention contains other detergent enzymes besides the lipase, such as protease, amylase or cellulase. Alkaline Bacillus proteases are preferred due to their well-known efficiency as detergent proteases. As such enzymes the proteolytic enzyme ALCALASE(R) from NOVO INDUSTRI A/S, manufactured microbially by cultivation of Bacillus liceniformis, or the proteolytic enzymes SAVINASE(R) and ESPERASE(R), also from NOVO INDUSTRI A/S, manufactured according to US patent No. 3,723,250, can be used. The mixed enzymatic additive can be prepared either by mixing a previously prepared granulate of proteinase with a previously prepared granulate of lipase, or by mixing a concentrate of proteinase with a concentrate of lipase and then introducing this mixture into a granulating device, together with the usual granulating aids.

Protease is nowadays a common detergent ingredient, and as will be shown later, lipases of the invention are excellently compatible in detergent solution with important detergent proteases, such as those mentioned above. If both lipase and protease are to be added to a detergent it may be convenient to use them in the form of a mixed additive.

In a specially preferred embodiment of the enzymatic detergent additive according to the invention, the proteolytic activity is between about 0.5 and about 3.0 Anson Units/g of additive. In this manner, a convenient proteolytic activity is generated in the washing solution when the detergent additive is added to the detergent in an amount of 0.2 - 2 g/100 g of detergent, and when the detergent is added to the washing solution in an amount of 0.5 - 20 g of detergent/l of washing solution. The well-known Anson hemoglobin method for proteolytic activity is described in Journal of General Physiology, 22, 79-89 (1959).

Detergent

In accordance with the previously indicated embodiments of the additive according to the invention, the detergent according to the invention may be a powder or a liquid and may optionally include other detergent enzymes, such as protease, amylase or cellulase, either in the same additive or as separate additives.

In a specially preferred embodiment of the detergent according to the invention, the detergent contains the

enzymatic detergent additive according to the invention in an amount of between 0.1 and 5% w/w, more preferably in an amount of 0.2 -2% w/w. In this manner, a reasonable balance between enzyme action and the action of the other detergent ingredients is generated.

The detergent is typically used in concentrations of 0.5 - 20 g/l of washing solution, and suitable lipase activity in the washing solution is 1,000 - 10,000 LU/l, more preferably 1,000 - 5,000 LU/l. Accordingly, in a preferred embodiment the lipase activity in the detergent is 50 - 20,000 LU/g, more preferably 50 - 10,000 LU/g, still more preferably 250 - 2,000 LU/g and most preferably 500 - 2,000 LU/g of detergent.

As mentioned above, the preferred lipase in the additive is above 10,000 LU/g, and this is added to the detergents in amounts of preferably 0.1 - 5% w/w and more preferably 0.2 - 2% w/w. Accordingly in another preferred embodiment the lipase activity in the detergent is 10 - 500 LU/g, more preferably 20 - 200 LU/g of detergent.

In a specially preferred embodiment, the detergent of the invention comprises other detergent enzymes besides the lipase, most preferably a protease. Preferred detergent proteases are those already mentioned. Lipase and protease may be added to the detergent either separately or in the form of a mixed additive. As already mentioned, proteases are commonly used in detergents, and lipases of the invention show a remarkable stability in detergent solution with the commercially important proteases. In accordance with the above-mentioned preferred ranges for protease activity in the additive and for the amount of additive in the detergent, we prefer a protease activity in the detergent of 0.0005 - 0.15 AU/g, more preferably 0.001 - 0.060 AU/g, still more preferably 0.003 - 0.025 AU/g and most preferably 0.006 - 0.010 AU/g of detergent.

In a specially preferred embodiment of the detergent according to this invention, the surface active material comprises 30-100% anionic and 0-70% non-ionic surfactant, most preferably 50-100% anionic and 0-50% non-ionic surfactant. Detergency of lipase of this invention is specially pronounced in detergents with a high content of anionics, such as LAS (linear alkyl benzene sulfonate).

Washing method

In a specially preferred embodiment of the washing process according to the invention, the washing solution contains the detergent according to the invention in an amount of between 0.5 and 20 g/l of washing solution. In this manner, a convenient lipase activity is generated in the washing solution, i.e. typically between 1,000 and 10,000 LU/l of washing solution, preferably between 1,000 and 5,000 LU/l of washing solution.

BRIEF DESCRIPTION OF THE DRAWINGS

Figs. 1 - 4 show pH-activity curves, figs. 1 - 2 are for DSM 3819 lipase, and figs 3 - 4 for DSM 1800 lipase. Figs. 1 and 3 are by the tributyrine method, and figs. 2 and 4 by the olive oil/PVC method.

EXAMPLES

Lipase activity

The method is based on hydrolysis of tributyrin in a pH-stat. 1 LU (Lipase Unit) is the amount of enzyme which liberates 1 µmol titratable butyric acid per minute at 30°C, pH 7.0 with gum arabic as an emulsifier. Further details are given in Novo analytical Method AF 95/5, available on request.

EXAMPLE 1

Lipase from H. lanuginosa DSM 3819

Each of fourty 500 ml shake flasks with 200 ml PL-IC medium (composition indicated below) in each were inoculated with 0.2 ml of a spore suspension prepared on the basis of slants with H. lanuginosa DSM 3819 grown on YPG-agar (composition indicated below) for 5 days at 45°C. The thus inoculated shake flasks were shaken 3 days at 45° C at 240 rpm. At this stage the lipase activity of the accumulated broth (6.7 litres) was 104 LU/ml. The cells were removed by centrifugation at 4000 rpm for 25 minutes. 5.9 litres of supernatant was obtained. The supernatant was filtered through a 10 µ nylon filter cloth prior to 8 x concentration by ultrafiltration on Pellicon UF Cassette system (membranes with NMWL of 10,000, NMWL being an abbreviation of nominal molecular weight limit).

The UF-concentrate (final volume of 740 ml) was converted to a crude powder by freeze-drying. This crude powder exhibited a lipase activity of 13,310 LU/g.

Composition of YPG-agar was as follows:

Yeast extract, Difco	4	g/l
Glucose	15	g/l
K_2HPO_4	1	g/l
$MgSO_4, 7H_2O$	0.5	g/l
Agar	20	g/l

Autoclaved at 121°C for 40 minutes.
Composition of PL-1c medium was as follows:

Peptone	15	g/l
Tween-80	18	g/l
$MgSO_4$, $7H_2O$	2	g/l
$CaCl_2$, $2H_2O$	0.1	g/l
Nalco-10	2	g/l
pH before autoclaving	6.0	

Autoclaved at 121°C for 40 minutes.

EXAMPLE 2

Lipase from other Humicola strains

Strain DSM 4111 was fermented on medium PL-1c, strain DSM 4109 on medium GT, strain DSM 4110 on medium GTS-1, and strain DSM 1800 on medium LR-8ST, essentially as described in Example 1.

Composition of fermentation media:		GT	GTS-1	LR-8ST
Yeast extract (65% dry matter)	g/l	22.5	15	0
Pharmamedia	-	0	0	50
Tween 80	-	18	5	5
Span 80	-	0	5	5
$MgSO_4$, $7H_2O$	-	2	2	0.5
$CaCl_2$, $2H_2O$	-	0.1	0.1	0
K_2HPO_4	-	0	0	5
$NaNO_3$	-	0	0	1
Nalco-10	-	5	2	0
pH before autoclaving		~6.5	~6.0	~7.0

Recovery from the culture broths was performed essentially as described for DSM 3819 in Example 1. In the instance of lipase from DSM 4109, an additional purification step was performed prior to freeze-drying: the UF-Concentrate was precipitated with acetone whereafter the precipitate was redissolved in water and freeze-dried. The resulting freeze-dried powders exhibited the following lipase activities:

Strain	DSM 4111	DSM 4109	DSM 4110	DSM 1800
LU/g	32,000	211,000	6,600	1,800

Washing method

The test material employed for washing trials was cotton fabric (with a surface weight corresponding to around 1.2 g/50 cm^2) impregnated with olive oil (Sigma 0-1500). The swatches were produced simply by dropping 50 or 85 µl (as indicated) of olive oil heated to 50-60° C on the centre of each test swatch (7 x 7 cm) by means of a micropipette. After oil application, the swatches were aged at room temperature for about 2 days.

The lipase preparations from Examples 1 and 2 were used, each identified by the strain number.

Also, as a comparison, a lipolytic powder on the basis of Fusarium oxysporum, obtained as described in Example 23 in European patent publication No. 0 130 064, and representing the most efficient known lipolytic detergent additive, was used. The activity of the Fusarium oxysporum lipase preparation was 90,000 LU/g.

The lipases were evaluated in washing tests in a Terg-O-Tometer test washing machine. The Terg-O-Tometer test washing machine is described in Jay C. Harris, Detergency evaluation and testing, Interscience Publishers Ltd., 1954, pages 60-61.

The washing trials were carried out under the following conditions:

Agitation 100 rpm
Water hardness 18°German hardness (tap water) unless otherwise noted
Cloth/liquid ratio 7 swatches/1000 ml
Rinsing 15 min in running tap water

The amount of oil contained in 7 swatches with 85 µl each is approx. 535 mg (density 0.90).

After rinsing, the swatches were air dried. The residual oil content in the swatches was determined by Soxhlet extraction with n-hexane for 5 hours followed by gravimetric determination of residual matter.

The composition of the residual oil was analyzed by the TLC-FID (TLC/FID is an abbreviation for thin layer chromatography/flame ionization detector, the method being described in Lipids, vol. 18, No. 10 (1983), page 732) method using a Iatroscan TH-10 (Iatron Lab. Inc., Tokyo) combined with a Chromatocorder II (System Instruments Co., Ltd., Tokyo) computing integrator under the following conditions:

Stationary phase Chromarod S-II (Iatron)
Mobile phase Hexane/chloroform/ acetic acid (60:50:2 v/v/v)
Hydrogen flow rate 160 ml/min
Air flow rate 2000 ml/min
Scan speed 30 sec per scan

Samples for the TLC-FID analysis were prepared as follows. After gravimetrical determination of residual matter the dried extract was redissolved in 20 ml of hexane and 5 ml of an internal standard (lithocholic acid, 12.5 mg/ml) dissolved in ethanol was added. 1 µl of sample was used for each analysis.

Based on standard curves for triolein, diolein, monoolein, and oleic acid the relative composition (% w/w) of the residual oil was calculated.

The number of unhydrolyzed glyceride bonds in the residual oil was calculated using the following formula:

$$n = 10 \times M \left(\frac{3}{885} \times X_{TG} + \frac{2}{621} \times X_{DG} + \frac{1}{357} \times X_{MG}\right) \quad (\mu \text{ mole})$$

where X_{TG} is the percentage of triglyceride (% w/w)
X_{DG} is the percentage diglyceride (% w/w)
X_{MG} is the percentage monoglyceride (% w/w)
M is the residual amount of oil (mg)
885, 621, and 357 are the mole weights for triolein, diolein, and monoolein, respectively.

EXAMPLE 3

Effect of washing temperature

This example demonstrates the effect of the Humicola lanuginosa lipase (DSM 3819) in an anionic detergent at different wash temperatures.

Detergent composition: LAS (0.5 g/l), Na_2CO_3 (1.0 g/l)
Washing time: 20 min.
Lipase dosage: 3000 LU/l
pH: 9.5
Soiling: 85 µl olive oil

LAS is a linear alkyl benzene sulfonate (Nansa HS80/S, Albright & Wilson), an anionic surfactant.

Temp.		Without lipase	Fusarium oxysporum	Humicola lanuginosa
30°C	Residual oil (mg)	185	187	165
	n (μ mole)	590	571	360
50°C	Residual oil (mg)	187	192	157
	n (μ mole)	606	628	432

EXAMPLE 4

Effect of washing time

In this example the effect of the Humicola lanuginosa lipase (DSM 3819) is demonstrated using different washing times.

Detergent composition: LAS (0.5 g/l), Na_2CO_3(1.0 g/l)
Temperature: 30°C
Lipase dosage: 3000 LU/l
pH (initial): 9.5
Soiling: 85 μl olive oil

Washing time (min.)		Without lipase	Fusarium oxysporum	Humicola lanuginosa
20	Residual oil (mg)	185	187	165
	n (μ mole)	590	571	360
40	Residual oil (mg)	177	167	128
	n (μ mole)	568	526	246
60	Residual oil (mg)	141	147	93
	n (μ mole)	465	454	153
90	Residual oil (mg)	139	135	78
	n (μ mole)	431	419	111

EXAMPLE 5

Effect of water hardness on washing

This example shows the influence of water hardness on the detergency of Humicola lanuginosa lipase (DSM 3819). The hardness (°GH = °German hardness) was adjusted by mixing tap water and distilled water.

Detergent composition: LAS (0.5 g/l), Na_2CO_3 (1.0 g/l)
Temperature: 30°C
Washing time: 20 min.
Lipase dosage: 3000 LU/l
pH (initial): 9.5
Soiling: 85 μl olive oil

Hardness °GH		Without lipase	Fusarium oxysporum	Humicola lanuginosa
0	Residual oil (mg)	254	244	242
	n (µ mole)	820	752	627
6	Residual oil (mg)	210	192	173
	n (µ mole)	670	595	415
12	Residual oil (mg)	182	179	170
	n (µ mole)	579	548	405
18	Residual oil (mg)	185	187	165
	n (µ mole)	590	571	360

EXAMPLE 6

Effect of lipase dosage on washing

This example shows the influence of dosage of H. lanuginosa lipase on washing performance, using acetone fractionated lipase powder from DSM 3819.

Acetone fractionation was done on 10g of the crude powder prepared in Example 1, dissolved in water to 104 ml total volume. Final acetone concentration was 45% by volume. After freeze-drying the re-dissolved acetone precipitate, 0.629 g with an activity of 160,960 LU/g was obtained. This was used in the following washing tests.

Detergent composition: LAS (0.5 g/l), Na_2CO_3 (1.0 g/l)
Washing time 20 min
Temperature. 30°C
pH (initial): 9.5
Soiling: 85 µl olive oil

The soiled swatches used were a different batch from Examples 2-5, so results are not directly comparable.

Lipase dosage, LU/ml	0	500	1500	3000	6000	10,000
Residual oil (mg)	232	209	202	202	194	176
n (µ mole)	761	558	521	471	437	363

EXAMPLE 7

Comparison of Humicola lipases in washing

This example compares the washing effect obtained with lipase from H. lanuginosa (DSM 4109), H. brevis var. thermoidea (DSM 4111), H. brevispora (DSM 4110) and H. insolens (DSM 1800).

Detergent composition:	LAS	0.50 g/l
	Tallow soap	0.05 -
	Alcoholethoxylate (C_{12-14},6EO)	0.10 -
	Alcoholethoxylate (C_{16-18},30EO)	0.02 -
	Zeolite	1.20 -
	Na_2CO_3	0.50 -
	Sodium metasilicate	0.10 -
	EDTA (titriplex III)	0.01 -
	Na_2SO_4	2.00 -
Temperature:	30°C	
Washing time:	20 min	
Lipase dosage:	6000 LU/l	
pH:	9.5	
Soiling:	50µl olive oil)	

Lipase preparation	n (µmoles)
none	546
H. lanuginosa	454
H. brevis var. thermoidea	468
H. brevispora	484
H. insolens	350

EXAMPLE 8

Protease stability of Humicola lipases

The excellent stability of Humicola lipases in detergent solutions containing proteolytic enzymes is demonstrated below.

A Humicola lanuginosa preparation (DSM 4109) is compared to the Fusarium oxysporum lipase used in previous examples and to the commercial lipase preparation, Amano P (Amano Pharmaceutical co. Ltd., Nagoya, Japan), which is produced by Pseudomonas fluorescens.

The alkaline Bacillus proteases ALCALASE, SAVINASE and ESPERASE were used. These are commercial detergent proteases from Novo Industri A/S, Denmark.

The proteolytic activity was determined with casein as the substrate. One Casein Protease Unit (CPU) is defined as the amount of enzyme liberating 1 mM of primary amino groups (determined by comparison with a serine standard) per minute under standard conditions, i.e. incubation for 30 minutes at 25°C and pH 9.5. A 2% (w/v) solution of casein (Hammersten, supplied by Merck A.G., West Germany) was prepared with the Universal Buffer described by Britton and Robinson (Journ. Chem. Soc. 1931, p. 1451) adjusted to pH 9.5.

Detergent: 1.3 g/l of a non-phosphate powder containing 25% surfactant (alphaolefin sulphonate (AOS) and linear alkyl-benzene sulphonate (LAS)), sodium sulphate, zeolite, sodium silicate and optical brightener.
Water hardness: 4.5° German hardness
pH: 10.0 (adjusted)
Temperature: 25°C
Lipase activity (initial): 3000 LU/l
Protease activity: 0 or 0.05 CPU/l
Residual lipase activity (%):

1) Protease: SAVINASE

Lipase	Incubation time (min)				
	0	15	30	60	90
Humicola lanuginosa	100	99	94	91	89
Fusarium oxysporum	100	32	14	3	-
Pseudomonas fluorescens	100	1	0	-	-

2) Protease: None

Lipase	Incubation time (min)				
	0	15	30	60	90
Humicola lanuginosa	100	101	99	102	96
Fusarium oxysporum	100	57	42	18	6
Pseudomonas fluorescens	100	94	94	88	90

Detergent: LAS 0.40 g/l
Alcoholethoxylate (Berol 065) 0.15 g/l
Tallow soap 0.15 g/l
Sodium tripolyphosphate 1.50 g/l
Sodium metasilicate 0.40 g/l
CMC 0.05 g/l
Na_2SO_4 2,10 g/l
Water hardness: 18° German hardness
pH: 9.5
Temperature: 30°C
Lipase activity: 3000 LU/l
Protease activity: 0 or 0.05 CPU/l

1) Protease: SAVINASE

Lipase	Incubation time (min)				
	0	15	30	60	90
Humicola lanuginosa	100	99	98	97	97
Fusarium oxysporum	100	5	0	-	-
Pseudomonas fluorescens	100	0	-	-	-

2) Protease: ALCALASE

Lipase	Incubation time (min)				
	0	15	30	60	90
Humicola lanuginosa	100	98	97	95	95
Fusarium oxysporum	100	24	4	0	-
Pseudomonas fluorescens	100	18	0	-	-

3) Protease: ESPERASE

Lipase	Incubation time (min)				
	0	15	30	60	90
Humicola lanuginosa	100	97	96	96	98
Fusarium oxysporum	100	20	0	-	-
Pseudomonas fluorescens	100	0	-	-	-

4) Protease: None

Lipase	Incubation time (min)				
	0	15	30	60	90
Humicola lanuginosa	100	96	95	96	94
Fusarium oxysporum	100	30	10	0	-
Pseudomonas fluorescens	100	101	102	102	99

It is seen that the Humicola lipase of the invention is very stable in detergent solution with protease, in contrast to prior-art detergent lipases (Fusarium and Pseudomonas).

EXAMPLE 9

Stabilized liquid Humicola lipase preparations

Lipase stability in solutions with various stabilizers was investigated.

Lipase: Humicola lanuginosa (DSM 4109)

Storage temp: 30°C

pH: 7.0

Rodalon® was added to all preparations as a preservative (0.2 mg active matter per ml).

Results:

	1,2-propanediol (ml/ml)	Sorbitol (g/ml)	$CaCl_2.2H_2O$ (mg/ml)	INITITAL ACTIVITY (LU/ml)	Residual activity (%), days 0	2	13	29	49
1	0	0	0	4520	100	17	2	<1	<1
2	0.50	0	0	4520	100	93	63	35	34
3	0.50	0	3	4720	100	86	76	54	48
4	0	0.30	0	4880	100	57	10	<1	<1

These results demonstrate that 1,2-propanediol (MPG = mono propylene glycol) is an excellent stabilizer for Humicola lipase. The storage stability may be further improved by adding Ca salt. Sorbitol has a slight stabilizing effect.

A wash trial was carried out with a stabilized liquid lipase preparation with the following composition:

Humicola lanuginosa DSM 4109 lipase	5000 LU/ml
1,2-propanediol	50% v/v
Deionized water	50% v/v
$CaCl_2.2H_2O$	3 mg/ml

Detergent composition: LAS (0.5 g/l), Na_2CO_3 (1.0 g/l)
Temperature: 30°C
Washing time: 20 minutes
pH: 9.5
Soiling: 50 µl olive oil
Lipase dosage: 1 ml stabilized liquid preparation per liter wash liquor.

Results:

Lipase dosage LU/l	Residual oil (mg)	n (µmoles)
0	157	507
5000	145	418

EXAMPLE 10

Humicola lipase as dust-free granulate

A carrier granulate without enzyme was prepared essentially according to US patent 4,106,991 with the following composition:

15% cellulose fibres
4% titanium dioxide
5% yellow dextrin

10% sucrose
64% sodium sulphate
This granulate was sieved to obtain a 300-710 µm particle size.

30.8 g of this was wetted by 6.2 g of a 30% solution in ethanol of polyvinylpyrrolidon (PVP K30, product of GAF Corp., USA). After thorough mixing, 12.3 of freeze-dried H. lanuginosa DSM 4109 lipase (92,700 LU/g, prepared essentially as in Example 2) was added, and was thoroughly mixed. The granulate was dried (evaporation of ethanol) by air blowing (fluidization) at about 50°C. 300-850 µm particles were collected by sieving.

The granulate was then coated in three steps, as follows:
- 5% of polyethyleneglycol (MW 600)
- 11.25% of TiO_2/Mg silicate (4:1)
- 4% of polyethyleneglycol (MW 600)

The coated granulate was air-blown at 0.8 m/sec for 10 minutes to remove fine particles of coating material. Finally, the material was sieved again, and the 300-850 µm range was collected. A dust-free, off-white granulate was obtained.

Yield and activity were as follows:

freeze-dried powder	92,700 LU/g	12.3 g
un-coated granulate	21,100 -	45.0 -
coated granulate	18,200 -	

A washing trial was carried out with the freeze-dried powder and the granulate as follows:
Detergent composition: LAS (0.5 g/l), Na_2CO_3 (1.0 g/l)
Temperature: 30°C
Washing time: 20 minutes
Lipase dosage: 6000 LU/l
pH: 10.0
Soiling: 50 µl olive oil

Results:

Lipase preparation	n (µmoles)
none	515
freeze-dried powder	386
granulate	415

The features disclosed in the foregoing description, in the following claims and/or in the accompanying drawings may, both separately and in any combination thereof, be material for realising the invention in diverse forms thereof.

Claims

1. Enzymatic detergent additive the active component of which is a microbially produced lipase, wherein the lipase is producible by means of a strain of the genus Humicola, including the genus Thermomyces.

2. Enzymatic additive according to Claim 1, wherein the strain belongs to a thermophilic Humicola sp., including thermophilic Thermomyces sp.

3. Enzymatic detergent additive according to Claim 1, wherein the strain belongs to H. lanuginosa (Griffon and Maublanc) Bunce, H. brevispora Subrahmanyam and Thirumalachar, H. Brevis var. thermoidea Subrahmanyam and Thirumalacher or H. insolens Cooney & Emerson.

4. Enzymatic detergent additive according to Claim 3, wherein the strain is H. lanuginosa DSM 3819.

5. Enzymatic detergent additive the active component of which is a microbially produced lipase, wherein the lipase cross-reacts immunologically with the lipase from Humicola lanuginosa strain DSM 3819.

6. Enzymatic detergent additive according to Claims 1 to 5, wherein the additive is provided as a non-dusting granulate or a liquid.

7. Enzymatic detergent additive according to Claim 6, wherein the additive is provided as a liquid containing an enzyme stabilizer.

8. Enzymatic detergent additie according to Claim 7, wherein the stabilizer is propylene glycol.

9. Enzymatic detergent additive according to Claims 1 to 8, wherein the lipase activity is above about 10,000 LU/g of additive.

10. Enzymatic detergent additive according to Claims 1 to 9, wherein the additive besides the lipase

contains a proteolytic enzyme.

11. Enzymatic detergent additive according to Claim 10, wherein the proteolytic activity is between about 0.5 and about 3.0 Anson units/g of additive.

12. Enzymatic detergent additive according to claim 10 or 11, wherein the proteolytic enzyme is an alkaline Bacillus protease.

13. Enzymatic detergent additive according to Claims 12, wherein the proteolytic enzyme is manufactured microbially by means of Bacillus licheniformis or is manufactured according to US patent No. 3,723,250.

14. Detergent comprising an enzymatic additive, the active component of which is a microbially produced lipase, wherein the enzymatic detergent additive is the enzymatic additive according to Claims 1 to 13.

15. Detergent according to Claim 14, provided as a powder or a liquid.

16. Detergent according to Claims 14 or 15, wherein the detergent contains the enzymatic detergent additive according to Claim 1 to 12 in an amount of between 0.1 and 5% w/w.

17. Detergent according to Claims 14 to 16, wherein the surface active material comprises from 30 to 100 weight % of anionic surfactant and from 0 to 70 weight % of non-ionic surfactant.

18. Detergent according to Claims 14 to 17, wherein the lipase activity is between 50 and 20,000 LU/g of detergent.

19. Detergent according to Claims 14 to 18, wherein the detergent includes a protease.

20. Detergent according to Claim 19, wherein the protease activity is between 0.0005 and 0.15 AU/g of detergent.

21. Washing process, wherein the detergent is the detergent according to Claims 14 to 20 and the pH is between 7 and 12.

22. Washing process according to Claim 21, wherein the washing solution contains the detergent according to Claims 14 to 20 in an amount of between 0.5 and 20 g per litre of washing solution.

Fig.1

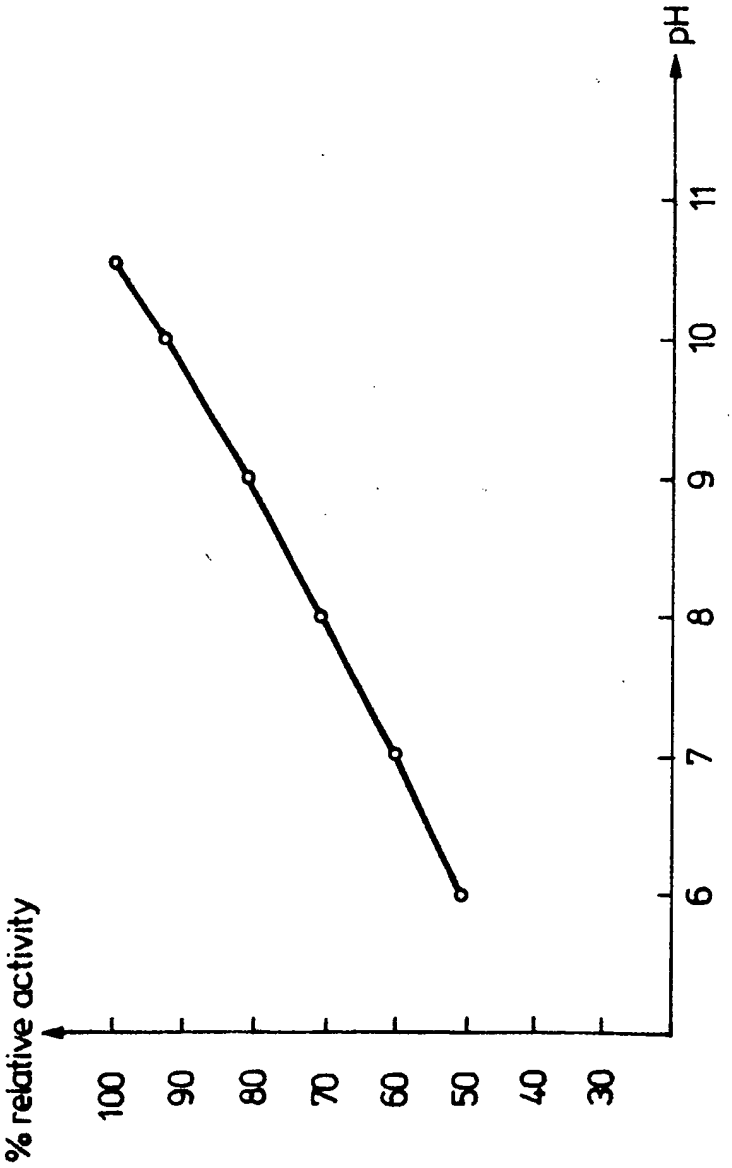

Fig.2

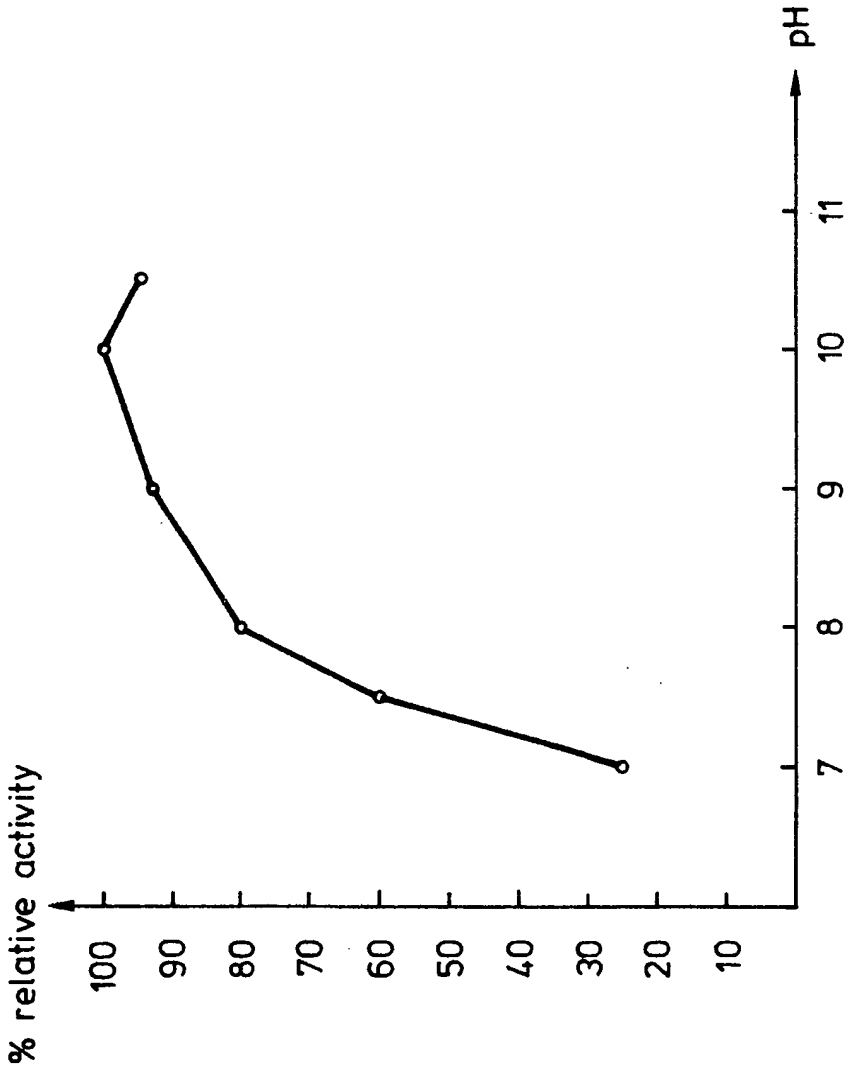

Fig. 3

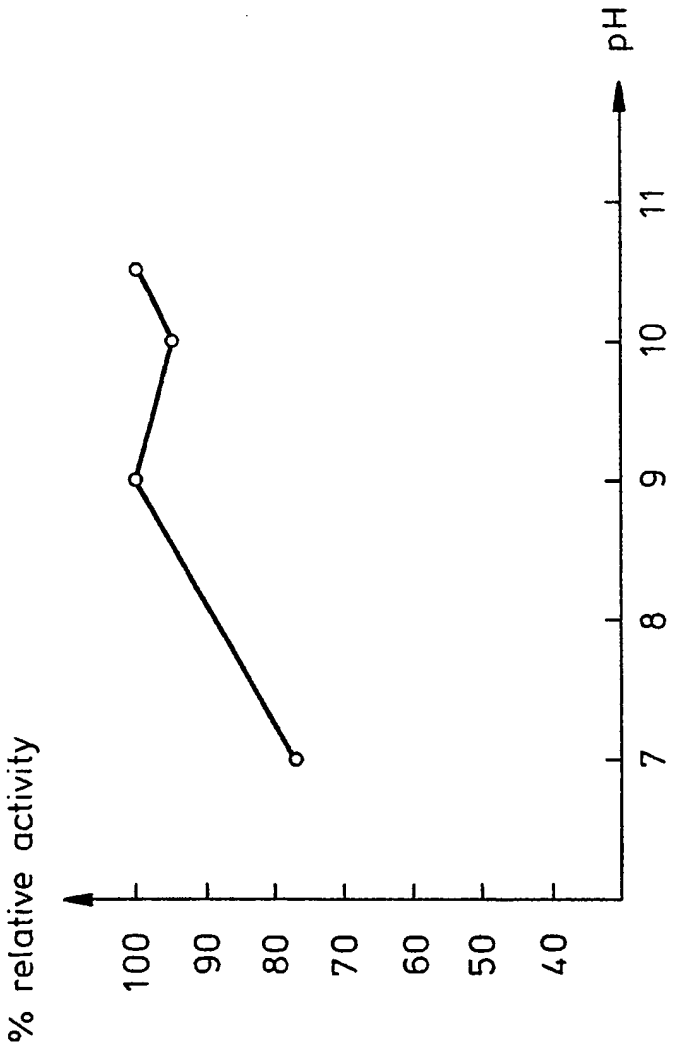

Fig. 4